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The Study of Cytostatic Effect on MCF-7 Cells of the Alcohol Extract of *Rhodiola Algida* Var. *Tangutica*

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Abstract

Aims: To study the anti-tumor effect on MCF-7 cells and the related mechanism of the alcohol extract of *Rhodiola algida* Var. *tangutica*. **Methods:** The effective constituent of the extract was detected with the high efficiency liquid chromatography. The survival rate of MCF-7 cells was evaluated by MTT test. The morphological change of cells was observed with optical microscope. The apoptotic influence of the extract on cells was researched with Annexin V-FITC/PI kit and flow cytometry. **Results:** The main constituent of the extract contained the salidroside and tyrosol. The extract caused decrease in the survival of MCF-7. The results of optical microscope showed the vacuole and apoptotic body in MCF-7 cells. The FCM analysis showed that lower concentrations of the extract caused much apoptotic cells and higher concentrations led to much necrotic cells. **Conclusion:** The extract of *Rhodiola algida* Var. *tangutica* inhibits division of MCF-7 cells and the cytostatic and antiproliferative effect was related to apoptosis induction mechanism.

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Keywords: extract; *Rhodiola algida* Var. *tangutica*; MCF-7 cells; antiproliferative; apoptosis;

1. Introduction

Rhodiola rosea is one of medicinal plants having stimulating and adaptogenic properties [1]. Recently experimental studies have revealed the *Rhodiola rosea* L showed the cytostatic properties on S180 Solid tumor in vitro [2]. *Rhodiola algida* Var. *tangutica* (Crassulaceae) has been used for a very long time in Qinghai and Tibetan folk medicine. In spite of was used in some tibetan medicinal prescriptions for tumor

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therapy, little is known about the anticancer action of the *Rhodiola algida* Var. *tangutica*. The aim of this report is to investigate the antitumor activities of the 75% alcohol extract of *Rhodiola algida* Var. *tangutica* and the mechanism of its action.

2. Materials and methods

2.1. Extract preparation

The rhizomes of *Rhodiola algida* Var. *tangutica* were obtained from Yushu (4700m), Qinghai. The plant material was dried. 10 g of ground dry raw material was dipped in 60 ml of 75% alcohol. Extraction was carried out in Soxhlet extractor and flasks on a rotary shaker. The extract was filtered, and the filtrate was evaporated at 35 °C at reduced pressure (20 mbar). The dry residue was dissolved in dimethyl sulphoxide (DMSO) whose final concentration was 2%. This solution was used for dilution with distilled water. During investigation, the following dilutions of the basic solution were used: 45, 90, 180, 225, 360 and 450 µg/ml, where the maximal concentration of DMSO in the obtained dilutions did not exceed 0.5%.

2.2. HPLC analysis

HPLC analysis was performed on a Waters system. The mobile phase consisted of methanol-A and 0.01 M H₃PO₄-B used in the following gradient elution. The flow rate was 1.0 ml/min and detection 220 nm. The standard substances of salidroside and tyrosol were analyzed in the same conditions.

2.3. Cell culture and MTT assay

Breast cells of the MCF-7 line were obtained from the institute of biochemistry and cell biology, Shanghai. The MCF-7 cells were cultured in DMEM (GIBCO) medium supplemented with 10% inactivated newborn calf serum, penicillin and streptomycin at 37°C in air supplemented with 5% CO₂. For cytological investigation, 104 cells per ml of DMEM medium were used. Cells were treated with different concentrations of the extract for 12, 24, 48 h. After each time of incubation, cells were collected for further MTT assay. The microscopic observation was detected with optical microscope

2.4. Apoptosis /necrosis assessed by flow cytometry

The mode of cell death via apoptosis and or necrosis produced by the extract treatment in MCF-7 cells was determined by flow cytometry after staining the cells with Annexin V-FITC and PI.

3. Results

3.1. HPLC analysis

Analysis of the retention times and UV-spectra of compounds present in *Rhodiola algida* Var. *tangutica* rhizome extract, and comparison of standard substances with the same data, showed the presence of salidroside and tyrosol. There were also unidentified compounds in the extract. (Fig.1). According to reports, the salidroside is present in roots more than 5 years old and for therapeutic use, roots older than 5 years are better [3].

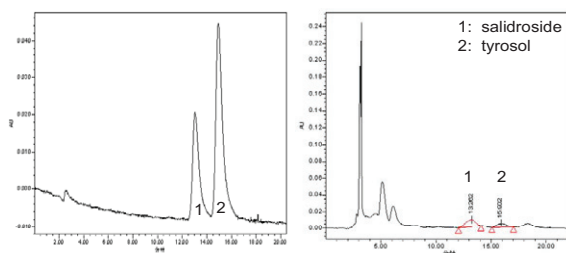


Fig.1 HPLC result of the alcohol extract of *Rhodiola algida* Var. *tangutica*

3.2. MTT assay

The result of the MTT assay is presented in table.1. Incubation of MCF-7 cells with the extract of *Rhodiola algida* Var. *tangutica* showed a significant dose-dependent and time-dependent decrease in the cell viability. The lower concentration narrowly decreased the survival of MCF-7 cells for 24 h and 48 h of incubation. Treatment with the highest concentrations of the extract (360 and 450 μ g/ml) markedly reduced survival and reduced it to almost 0 after 48 h of incubation.

Table .1 Cytotoxic effect of extract on MCF-7 cells assessed by MTT assay. Cells were treated 12, 24, 48 h and MTT assay was carried out.

Concentration (μ g/ml)	survival (%) of 12 h	survival (%) of 24 h	survival (%) of 48 h
45	76 \pm 0.49	117 \pm 3.88	93 \pm 3.96
90	71 \pm 3.28	109 \pm 3.45	98 \pm 1.73
180	36 \pm 2.96	68 \pm 3.60	40 \pm 1.62
225	35 \pm 1.85	61 \pm 1.31	36 \pm 2.78
360	23 \pm 3.73	20 \pm 2.18	6 \pm 1.80
450	12 \pm 2.63	9 \pm 1.21	2 \pm 0.14

3.3. Microscopic observation

One of the characteristics of drug-treated cells was cell morphology change. For further research, both the control cells and cells treated with the extract for 48 h were observed under an inverted microscope (Fig. 2). Shown in Fig. 2 B, cells treated with 225 μ g/ml displayed drastic morphological changes; for example the cells shrank, became round and vacuolization. And cells treated with 450 μ g/ml, apoptotic bodies were formed (2C).

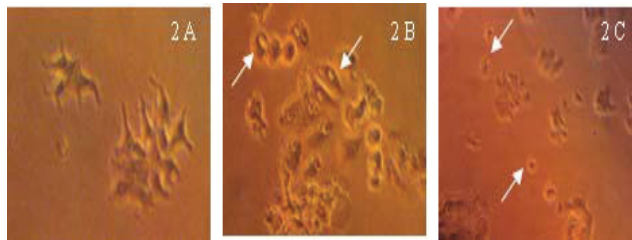


Fig. 2. Morphology changes of cells treated by the extract

3.4. Induction of apoptosis and/or necrosis assessed by flow cytometry

Externalization of phosphatidylserine (PS) to the outer leaflet from the inner leaflet of the plasma membrane is a hallmark of early apoptosis. The FITC labeled Annexin V binds to PS in presence of calcium ions, resulting in green fluorescence of apoptotic cells. In later stages of apoptosis or necrosis, PI enters the cells and bind to cellular DNA, resulting in red and intense green fluorescence with Annexin V. As shown in the Fig.3, cells treated with the lower concentration showed the present of Annexin V (+) (lower right quadrant) indicating the induction of early apoptosis. However, at higher concentration, there was a prominent rise of Annexin V(+)/PI (+) indicative of late apoptosis/necrosis. The appearance of late apoptotic/necrotic cells was predominantly seen at the higher concentrations of 450 $\mu\text{g/ml}$.

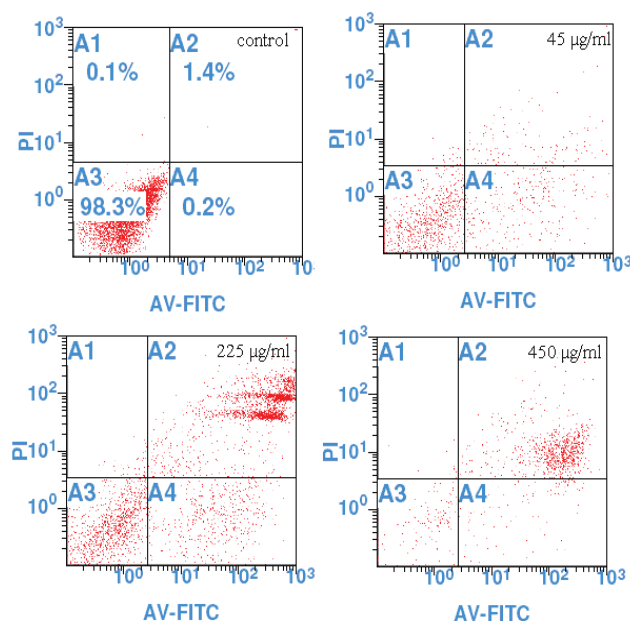


Fig.3. Flow cytometric analysis of apoptosis/necrotic cell death using Annexin V-FITC/PI staining of MCF-7 cells treated with the extract.

4. Discussion

According to recent reports, use of plant extracts is very effective in antitumor therapy, due to the supplementary or synergistic effect of particular compounds of the extract. The cytostatic effect of whole plant extracts on cancer cells is often much better than the effect of their particular biologically active compounds [4]. The results of our investigations determined that the main component of *Rhodiola algida* Var. *tangutica* contained salidroside and tyrosol. The 75% alcohol extract of *Rhodiola algida* Var. *tangutica* showed effective anti-tumor activities in vitro. The extract leads to induction of apoptosis and necrosis in MCF-7 cells and to a marked reduction of their survival. The cytostatic effect of 75% alcohol extract of *Rhodiola algida* Var. *tangutica* raises hope that it could be very promising for their use in supplementary anticancer therapy.

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